REMARKS

The claims have been amended substantively so as to require further consideration and/or search. For this reason, a Request for Continued Examination is being filed with this amendment.

This application is amended in a manner to place it in condition for allowance at the time.

Status of the Claims

Claims 19, 29, 30 and 31 have been amended so that the invention is directed to compositions including between 30 and 45% amylose content and includes hydroxypropylated starch. Support for the amendment may be found, for example, at page 14, lines 30-32, as well as Table 3 of the present specification, which illustrates unexpected results obtained by compositions including between 30 and 45% amylose content and hydroxypropylated starch.

Claim 28 has been amended to correspond to claim 19.

Claims 19-22 and 24-47 remain pending.

Claim Rejections-35 USC §103

Claims 19, 28, 38, and 39 stand rejected under U.S.C. § 103(a) as being unpatentable over HAASMAA US 2002 0032254 (HAASMAA) in view of LEUSNER US 4,431,800 (LEUSNER) and further in view of KIM US 6,123,963(KIM). Claims 19-22, 24-27, 29, 30,

32-38 and 40-43 were newly rejected under U.S.C. § 103(a) as being unpatentable over LYDZINSKI et al. U.S. 2003/0099692 (LYDZINSKI). Claims 31 and 44-46 stand rejected under U.S.C. § 103(a) as being unpatentable over LYDZINSKI in view of FUERTES US 6,469,161 (FUERTES).

These rejections are respectfully traversed for the reasons that follow.

The amended independent claims 19, 29, 30 and 31 relate to a

> *"A film-forming starchy composition for the film coating of solid forms or for the preparation of films, wherein said composition exhibits an amylose content of between <u>30</u> and 45%, this percentage being expressed by dry weight of starch present in said composition, and wherein said composition comprises at least one <u>hydroxypropylated legume starch</u>."*

Claims 19, 28, 38, and 39 are unobvious over HAASMAA in view of LEUSNER and further in view of KIM:

HAASMAA describes a starch dispersion containing a modified starch ester dispersed in a liquid phase wherein the degree of substitution (DS) of the starch ester is greater than 1.5 (see claim 1).

HAASMAA is completely silent about suitable amylose contents.

Moreover, HAASMAA does not lead one to the selection of a legume starch. HAASMAA provides an exhaustive list of starch sources (see page 2, right column, lines 3-5), which includes

barley, potato, wheat, oat, pea, corn, tapioca, sago, rice, or a similar rubber-bearing or rain plant. However, HAASMAA appears to prefer barley starches, or at least cereal starches, as the Examples only utilize barley starches.

Even if one would have randomly selected pea starch from the large variety of starch sources provided by HAASMAA one would not have necessarily obtained an amylose content between 30 and 45%, as evidenced by HOOVER et al. (enclosed in the Appendix). HOOVER the amylose content depends on the <u>type</u> of pea. According to Table 2 on page 83, wrinkle pea may have 62.8-75.4% amylose and smooth pea may have 32.5-33% amylose.

Thus, HAASMAA completely fails to teach or suggest the claimed <u>30-45% amylose content</u> of the film-forming composition in combination with the use of a <u>hydroxypropylated legume</u> starch.

LEUSNER and KIM are unable to completely remedy these deficiencies of HAASMAA for reference purposes.

LEUSNER relates to a method for hydroxypropylating starch. In column 1, lines 23-24 it is mentioned that hydroxypropylation decreases the tendency towards retrogradation of the starches. LEUSNER is silent about starch sources.

As to the decreased tendency towards retrogradation of hydroxypropylated starches, Applicant has shown that decreased retrogradation is often only obtained in combination with high processing temperatures. This however creates other drawbacks,

such as decreased flavor impact of the final film (see example 2 of the filed specification, page 22, lines 22-31).

KIM teaches that conventional methods for coating tablets, granules, pellets, crystals, and capsules include coating in a fluidized bed and dip-coating (column 6, lines 58-65). However, KIM does not teach any film forming composition as presently claimed.

Furthermore, the combination HAASMAA, LEUSNER and KIM fails to suggest the superior results of the claimed invention, as demonstrated in Table 3 of the present specification:

TABLE 3

Starch	Type	Viscosity at 25° C. and 10% SC (mPa · s)	Strength of the film	Smooth appearance	Nonsticky feel	Absence of agglomerates during coating	Coating appearance
Pea starch (35/39% amylose)	Hydroxypropylated (DS = 0.2)	++ (270)	+++	++	+++	+++	+++
	Hydroxypropylated (DS = 0.2) fluidification-treated	+++ (126)	+++	+++	+++	+++	+++
	fluidification-treated Acetylated (DS = 0.021)	+++ (24)	+	+++	+++	++	+
Amylose-rich corn (70%)	Pregel Hydroxypropylated (DS = 0.21)	0 (>500)	+++	++	+++	+	+++
Manioc, 20% amylose	Hydroxypropylated (DS = 0.2)	0 (>500)	–	–	–	+++	–
Waxy corn, 21% amylose	Pregelatinized	(421)	nd	–	–	0	nd
Mixture of starches comprising 42% amylose	Native Amylose-rich waxy corn Hydroxypropylated (DS = 0.10)	++ (246)	++	++	+++	++	+++

As is clearly demonstrated above, legume starches falling within the claimed amylose content of 30-45% and being hydroxypropylated, optionally fluidifaction-treated, provided superior film properties compared to, for example, corn starches as also disclosed by HAASMAA. Thus, these results are unexpected in view of the teachings of HAASMAA, LEUSNER and KIM.

Therefore, the combination of HAASMAA, LEUSNER and KIM cannot render obvious the claimed invention, and withdrawal of the rejection is respectfully requested.

Claims 19-22, 24-27, 29, 30, 32-38 and 40-43 are unobvious in view of LYDZINSKI:

LYDZINSKI describes a film forming composition comprising a modified starch (see [0005]). The starch may be corn, pea, potato, sweet potato, banana, barley, wheat, rice, sago, amaranth, tapioca, arrowroot, canna, sorghum, and waxy or high amylase varieties thereof (see lines 1-5 of [0009]). The modification may be selected from a large variety of possible physical, chemical, and/or enzymatic modifications (see [0011] to [0013]), including hydroxypropylation.

The examples of LYDZINSKI, however, are limited to modified high amylose or waxy corn starches and native tapioca starch. None of the modified corn starches has an amylose content of between 30 and 45 % by dry weight as claimed. That is, high amylose starch has an amylose content of 70% (see [0039]),

and the waxy corn starches have an amylose content of at most 5 % (see [0040], [0042] - [0045], and [0009], which specifies that waxy starches have an amylopectin content of at least 95% and thus an amylose content of at most 5%).

Thus, LYDZINSKI fails to disclose or suggest a legume starch, a 30-45% amylose content and hydroxypropylation in combination.

Indeed, even if one were to randomly select a hydroxypropylated pea starch based on the broad disclosure of LYDZINSKI, one would not obtain the necessarily obtain the claimed invention, or the superior results.

As evidenced by HOOVER et al. (enclosed in the Appendix), the amylose content depends on the <u>type</u> of pea. According to Table 2 on page 83, wrinkle pea may have 62.8-75.4% amylose and smooth pea may have 32.5-33% amylose.

Moreover, as evidenced by Table 3 of the present specification and provided above, the claimed invention results in film properties that are superior and unexpected to those which are suggested by LYDZINSKI, especially the examples of LYDZINSKI.

The claimed invention provides, for example, a preferred viscosity, whereas the viscosities of compositions comprising waxy or amylose rich hydroxypropylated starches are significantly higher. Due to these high viscosities waxy or amylose rich hydroxypropylated starch based compositions as those

of LYDZINSKI cannot be applied to tablets by spraying under cold conditions (see example 1 on pages 16-21).

Furthermore, compositions based on modified amylose rich corn starch as those of LYDZINSKI require high processing temperatures in order to prevent retrogradation of the starch and to allow acceptable spreading of the film. These high temperatures lead to significant evaporation of flavoring compounds when preparing flavor strips. The aromatic impact of the resulting flavor strips is thus significantly decreased and they do no longer have the desired freshening function (see example 2, page 22, lines 22-31 of the specification as filed).

Regarding the use of waxy starches, films formed from compositions containing waxy starches as those of LYDZINSKI do not exhibit the necessary cohesion. As a result they crack upon drying (see example 2, page 22, lines 33-37 of the application as filed).

LYDZINSKI, however, is completely silent about these problems associated with the use of high amylose and waxy starches.

Accordingly, the results demonstrated by the claimed composition having amylose content of between 30 and 45% by dry weight of starch present in a composition and at least one hydroxypropylated legume starch, are unexpected in view LYDZINKI.

That is, the claimed composition overcomes the drawbacks of the compositions of LYDZINSKI discussed above.

Therefore, LYDZINSKI cannot render obvious the claimed invention, and withdrawal of the rejection is respectfully requested.

Claims 31 and 44-46 are unobvious over LYDZINSKI in view of FUERTES:

LYDZINSKI fails to disclose or suggest a composition having amylose content of between 30 and 45% by dry weight of starch present in a composition and at least one hydroxypropylated legume starch.

FUERTES relates to a chemical fluidification process for a starchy material. However, FUERTES is completely silent about specific film-forming compositions, in particular composition comprising hydroxypropylated or acetylated legume starch and having a certain amylose content.

Consequently, FUERTES does not remedy the shortcomings of LYDZINSKI for reference purposes.

Therefore, the claimed invention is not rendered obvious by the combination of LYDZINSKI and FUERTES, and withdrawal of the rejection is respectfully requested.

Conclusion

In view of the amendment to the claims and the foregoing remarks, this application is in condition for allowance at the time of the next Official Action. Allowance and passage to issue on that basis is respectfully requested.

Should there be any matters that need to be resolved in the present application, the Examiner is respectfully requested to contact the undersigned at the telephone number listed below.

The Commissioner is hereby authorized in this, concurrent, and future submissions, to charge any deficiency or credit any overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

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APPENDIX:

The Appendix includes the following item(s):

- HOOVER et al., "Composition, structure, functionality and chemical modification of legume starches: a review"

D13

Composition, structure, functionality, and chemical modification of legume starches: a review[1]

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HOOVER, R., and SOSULSKI, F. W. 1991. Composition, structure, functionality, and chemical modification of legume starches: a review. Can. J. Physiol. Pharmacol. 69: 79–92.

The major carbohydrate of the legume seed is starch, which represents up to 45% of the total seed weight. In recent years, substantial progress has been made in understanding the relationship between starch structure and functionality. However, these studies have been mainly on cereal and tuber starches. The present status of knowledge on the composition, structure, functionality, digestibility, and chemical modification of legume starches is reviewed. In addition present concepts of granule structure, gelatinization, retrogradation, and rheology are also reviewed. Future research needs in the area of legume starch chemistry are discussed.

Key words: legume starch, structure, functionality, modification, digestibility.

HOOVER, R., et SOSULSKI, F. W. 1991. Composition, structure, functionality, and chemical modification of legume starches: a review. Can. J. Physiol. Pharmacol. 69 : 79–92.

L'amidon est le principal glucide de la graine des légumineuses, représentant plus de 45% de son poids total. Depuis quelques années, notre compréhension de la relation entre la structure et la fonctionnalité de l'amidon s'est considérablement améliorée. Toutefois, les études ont porté principalement sur les amidons des tubercules et des céréales. On rend compte de l'état actuel de la connaissance sur la composition, la structure, la fonctionnalité, la digestibilité et la modification chimique des amidons des légumineuses. On révise aussi les concepts de structure, de gélatinisation, de rétrogradation et de rhéologie du grain. On discute des besoins de la recherche dans le domaine de la chimie de l'amidon des légumineuses.

Mots clés : amidon des légumineuses, structure, fonctionnalité, modification, digestibilité.

[Traduit par la Rédaction]

Introduction

Legumes are the dicotyledonous seeds of plants that belong to the family Leguminosae, which contains about 600 genera with 13 000 species. Legumes are grown in many countries including India, Sri-Lanka, Mexico, Pakistan, Bangladesh, Africa, and South America. Legume cultivation in Canada, unlike the countries listed above, are of recent origin. Production of legumes in Ontario (Canada) has ranged between 50 000 and 100 000 t annually, the value of the crop being estimated at over 160 million/year. About 20% of the crop is grown in other provinces of Canada for which statistics are not available.

Research on the structure and functional properties of the main starches of commerce such as wheat, corn, potato, and rice have resulted in their extensive utilization in industrial applications or food products. Furthermore, food uses and functional properties of legume flours, protein isolates and concentrates have also been studied extensively (Sosulski *et al.* 1976; Sosulski and Youngs 1979; Vose 1980; Sumner *et al.* 1981). However, legume starches, which form the main storage component (22–45%) of the seed, have not been subjected to intensive research and neither have they been used widely in the food industry. It is likely that their lack of availability and their high retrogradation rates (Hoover and Sosulski 1985*a*; Tjahjadi and Breene 1984) may have been the main causative factors. It was recently reported that the high retrogradation rates of legume starches could be reduced by chemical modification (Hoover and Sosulski 1985*b*; Hoover *et al.* 1988*b*) to levels that approach those of modified waxy maize starch. This, combined with their high thermal stability, should render legume starches advantages for use in the food industry. Furthermore, the resistance of legume starches towards hydrolytic enzymes have been of great interest to nutritionists, since they have been found to exhibit a lower glycaemic index than the cereals (Jenkins *et al.* 1980), thereby helping in the dietary control of diabetes as well as arterial disease.

This review summarizes the present knowledge on the composition, structure, functionality, and digestibility of native and modified legume starches, with a view to providing suggestions for needed research to improve the utilization of legume starches in the food industry.

Starch isolation

The isolation of starches from legume seeds is difficult owing to the presence of insoluble flocculent protein and fine fiber, which decreases sedimentation and cosettles with the starch to give a brownish deposit (Schoch and Maywald 1968; Hoover and Sosulski 1985*a*; Reichert and Youngs 1978). Legume starches are isolated using aqueous techniques as well as pin milling and air classification (Reichert and Youngs 1978; Schoch and Maywald 1968). Reichert and Youngs (1978) have shown that remilling and reclassification of air-

[1]This paper was presented at the symposium Starch in Human Nutrition, held June 19–21, 1989, University of Saskatchewan, Saskatoon, Sask., Canada, and has undergone the Journal's usual peer review.

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classified pea starch granules, followed by water washing, removes most of the residual protein attached to the starch granules, resulting in a protein content of 0.25% in the washed starch. Repeated filtration through polypropylene screens (202 and 70 μm) combined with alkali treatment (0.02% NaOH) has been shown to cause substantial reduction in the protein content of wet process extracted legume starches belonging to the species *Phaseolus vulgaris* (Hoover and Sosulski 1985*a*). The resulting protein content was found to range from 0.13 to 0.52%. Low protein lentil starches have been prepared by extraction of lentil seeds at pH 7.5–9.5 (Anderson and Romo 1976). Colonna *et al.* (1981*a*) showed that broad bean and smooth pea starches could be extracted in high yields (93.8–96.7%) from their respective flours after protein extraction at pH 9 using different sieving (200–60 μm) and washing conditions. The starches were found to be contaminated mainly by cell-wall polysaccharides (less than 4%). The protein in starches ranged from 0.3 to 0.4%.

Granule size and microscopic appearance

The size and shape of legume starch granules are shown in Fig. 1 and Table 1. The granule size is variable and ranges from 4 to 85 μm depending on the starch source. Most of the granules are oval, although, spherical, round, elliptical, and irregularly shaped granules are also found. When observed under a scanning electron microscope (SEM) the surfaces of all granules appear smooth with no evidence of any fissures (Fig. 1). Most of the legume starches are simple granules, the exception being wrinkled pea starch, which appears to be a mixture of simple and compound granules, the latter being composed of 3–10 individual subunits joined together (Colonna *et al.* 1982).

Proximate analysis and chemical composition

The proximate analysis and chemical composition of legume starches are illustrated in Table 2. The isolated starches had protein contents ranging from 0.10 to 1.12%. The ash content, reflecting contamination by fine fiber, ranged from 0.03 to 0.81%. The starches were generally characterized by a low lipid content (<0.6%), the exception being moth bean (0.87%). It is now recognized (Morrison 1981; Hoover *et al.* 1988*a*) that the most effective solvent system for the extraction of lipids, which are inside the starch granule, is hot *n*-propanol–water (3:1 v/v).

It is unclear as to whether internal lipids of native starch granules occur as inclusion complexes inside helical segments of amylose or they are simply entrapped between the starch components (Morrison 1981). However, Mercier *et al.* (1980) have demonstrated conclusively the formulation of amylose–lipid complexes during extrusion cooking. Hoover *et al.* (1988*a*) have shown, by the use of the above solvent system, the presence of internal starch lipids (0.09%) in field pea starch. Most of the data on the lipid content of legume starches (Table 2) have been obtained by the use of solvent systems that have proved to be ineffective in removing internal starch lipids. Therefore, in the light of these findings, the lipid content of all legume starches must be reexamined. Such a study is important, since lipid removal has been shown to alter starch functionality (Maningat and Juliano 1980; Goshima *et al.* 1985). Legume starches are characterized by a high amylose content (24–65%) (Table 2). Kawamura (1969) and Lineback and Ke (1975) have shown that determination of amylose content by calculation from the iodine affinity of the whole starch, assuming that pure amylose absorbs 200 mg iodine/g of amylose (Schoch 1964), gave consistently higher values than the colorimetric procedure described by McReady and Hassid (1943). A good agreement between these two methods was reported for horse bean starch but not for chick pea starch (Lineback and Ke 1975). Recently, Morrison and Laignelet (1983) developed an improved colorimetric procedure for the determination of apparent amylose (measured in the presence of amylose-complexing monoacyl lipids occurring naturally in the starch granule) and total amylose (measured on lipid-free starch). These authors have shown that the values for amylose content are low, if account has not been taken of lipid-complexed amylose. Therefore, the discrepancies that exist in the literature with respect to the amylose content of legume starches may be due to different methods used for amylose determination, varietal differences (Rosenthal *et al.* 1971; Shahen *et al.* 1978), physiological state of the seed (Banks *et al.* 1974), or to the amount of amylose in lipid complexed form during estimation (Morrison and Laignelet 1983).

Molecular structure

Most starches are a mixture of two polysaccharides, amylose and amylopectin. An intermediate fraction, which has properties closer to amylose with a lower molecular weight, is present in a proportion from 30 to 35% in starches of high amylose content (Colonna *et al.* 1982). The characterization of amylose and amylopectin has been carried out after solubilization and fractionation. Fractionation is effected by the addition of *n*-butanol, which interacts with the amylose helix, forming an insoluble complex; the amylopectin is then obtained from the supernatant. Amylopectin is the major component and contains branches macromolecules in which linear chains of (1 → 4)-α-D-glucose residues are connected through (1 → 6)-α-linkages (5–6%). Amylose, the minor component, consists of much longer linear chains consisting of α-D-glucopyranose residues linked by (1 → 4) bonds. However, a slight degree of branching has been found in amyloses from various starch sources, the degree of branching in amylose being very much less than in amylopectin. Although, much information concerning the fine structure of amylose and amylopectin has accumulated from studies on cereal and tuber starches, little information has been forthcoming on the fine structure of these components from legume starches. Physicochemical characteristics of some legume amyloses are presented in Table 3. The iodine binding capacity (IBC), limiting viscosity number (η), degree of polymerization (DP), and β-amyolysis limit for amylose from legume starches are in the range 16–22, 136–280, 1000–1900, and 79–86.9%, respectively. The molecular weights have been determined only on selected legume amyloses and they range from 165 000 to 312 000. The limiting viscosity number of legume amyloses are lower than those reported (Banks and Greenwood 1967) for cereals (330–435) and potato (410). Biliaderis *et al.* (1981) have shown that concurrent treatment of legume amyloses with β-amylase and pullulanase results in complete conversion of amylose into maltose, thus providing evidence of the existence of (1 → 6)-α-linkages in amylose and that these linkages constitute the only barrier to the action of β-amylase on amylose.

Biliaderis *et al.* (1981) and Colonna and Mercier (1984) have shown by gel chromatography and light scattering techniques, respectively, that amylopectin from legume starches have average molecular weights greater than 1.9×10^7. Iodine affinities, average chain lengths, and chain molar ratios

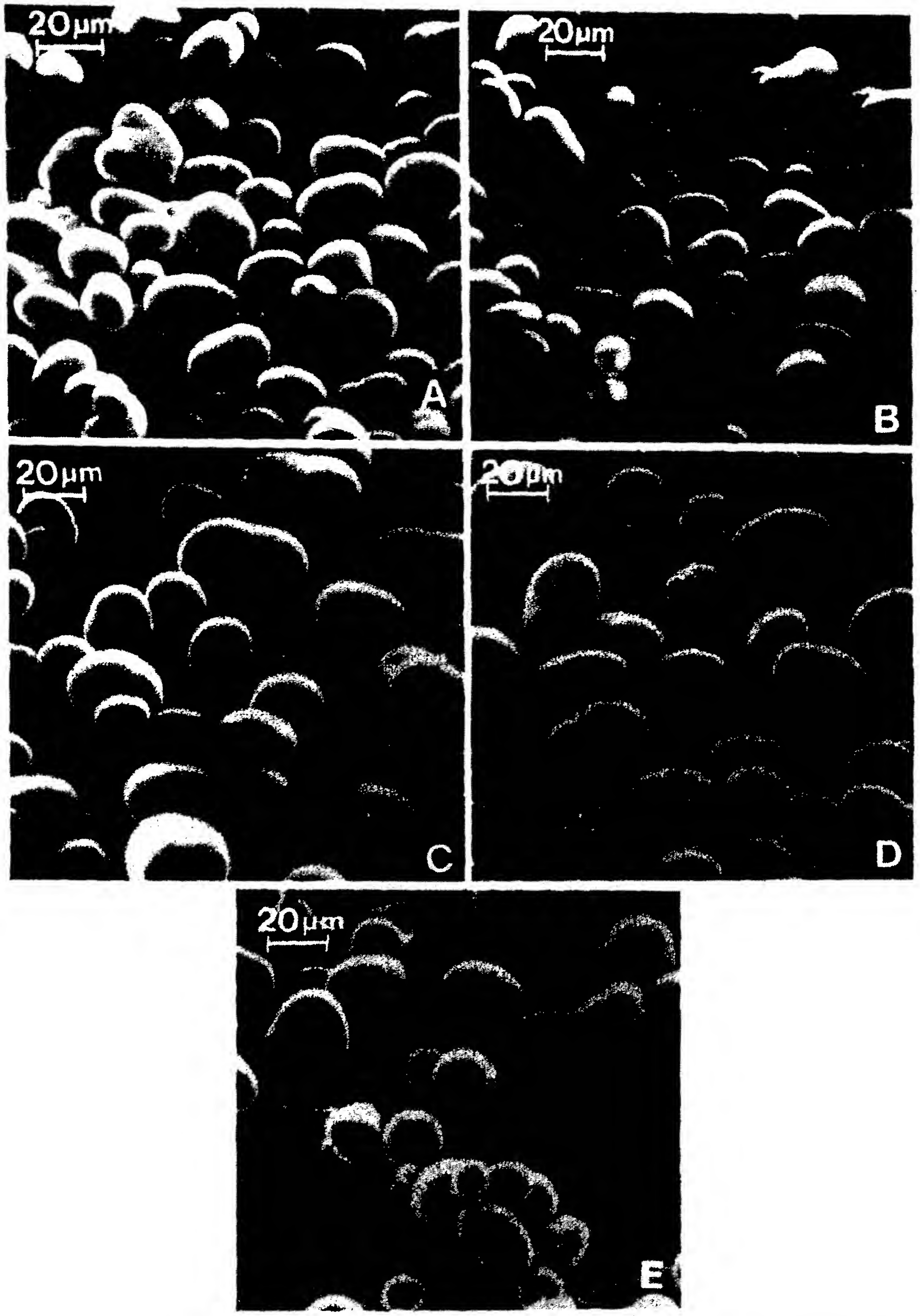

FIG. 1. SEM micrographs of native legume starches. (A) Black bean. (B) Pinto bean. (C) Kidney bean. (C) Northern bean. (E) Navy bean. (From Hoover and Sosulski (1985*a*), with permission.)

have been shown to range from 1.00 to 5.3, 20 to 26, and 4.2 to 9.6, respectively, for amylopectins from legume starches (Biliaderis *et al.* 1981). Colonna *et al.* (1981*b*) have shown that the fine structure of amylose and amylopectin of broad bean and smooth pea starches resemble those of normal cereal starches. However, the crystalline structure, studied by X-ray diffractometry and lintnerization, was found to be intermediate between the cereal and tuber types.

Starch source	Width (μm)	Length (μm)	Unspecified (μm)	Shape	Source
Kidney bean	16–42	16–60		Elliptical, oval	Kawamura 1969; Hoover and Sosulski 1985*a*
Northern bean	12–40	12–62		Oval, irregular, round	Hoover and Sosulski 1985*a*; Sathe and Solunkhe 1981
Navy bean	12–40	12–49		Oval, round elliptical	Hoover and Sosulski 1985*a*; Naivikul and D'Appolonia 1979
Black bean	8–34	8–55		Oval, spherical	Hoover and Sosulski 1985*a*; Lai and Varriano-Marston 1979
Mung bean	7–20	10–32		Oval, irregular, round	Kawamura 1969; Naivikul and D'Appolonia 1979
Pinto bean	10–30	12–48		Oval, irregular, round	Hoover and Sosulski 1985*a*; Naivikul and D'Appolonia 1979
Adzuki bean	20–55	24–70	15–45	Oval, kidney	Kawamura 1969; Tjahjadi and Breene 1984
Moth bean			6–28	Oval, round	Wankhede and Ramteke 1982
Faba bean	12–24	20–48		Oval, spherical	Naivikul and D'Appolonia 1979
Horse bean			6–31	Oval, irregular	Lineback and Ke 1975
Red bean			25–67	Oval, irregular	Lii and Chang 1981
Lablab bean			30	Oval, round, kidney	Rosenthal *et al.* 1971
Smooth pea			20–40	Oval, round	Vose 1977
Wrinkled pea			6–80	Round	Colonna *et al.* 1980
Black gram	7.5–27	7.5–28.5		Round, oval	Sathe *et al.* 1982
Chick pea			8–54	Oval, spherical	El Faki *et al.* 1983*a*; Lineback and Ke 1975
Cow pea			4–40	Oval, spherical	El Faki *et al.* 1983*a*; Tolmasquim *et al.* 1971
Horse gram			15–85	Oval, spherical	El Faki *et al.* 1983*a*
Lentil	15–30	10–36		Oval, round, ellipsoid	Naivikul and D'Appolonia 1979; Bhatty 1988

Colonna and Mercier (1984) have shown the presence of a branched intermediate fraction (18.9%) of low molecular weight (M_n = 25 100) in wrinkled pea starch. It was found to contain the same short (S, DP = 15) and long (L, DP = 45) linear chains as amylopectin, but the ratio S/L was 3.6, in contrast with 9.6 for wrinkled pea and 8.1 for smooth pea amylopectins. The presence of an intermediate fraction has not been reported in other legume starches.

Crystallinity of legume starches

X-ray diffractometry has been used to reveal the presence and characteristics of crystalline structures of starch granules (Katz and Van Itallie 1930; Sarko and Wu 1978). Legume starches have been shown to exhibit a "C" type (Hoover and Sosulski 1985*a*; Kawamura 1969; Sarko and Wu 1978; Colonna *et al.* 1981*b*; Lai and Varriano-Marston 1979) X-ray diffraction pattern, which is intermediate between the A (cereal) and the B type (tuber). The reasons for these differences are not properly understood. Hizukuri (1985) has suggested that slight differences in the chain length and chain profile of the constituent amylopectin molecules may be responsible for these differences in X-ray patterns. Hoover and Sosulski (1985*a*) have shown that most legume starches are characterized by two very strong intensity lines centered at 17.2 and 18.1° 2θ angles, which correspond, respectively, to interplanar spacings of 5.15 and 4.98 Å (1 Å = 0.1 nm). In contrast with other legume starches, wrinkled pea starch exhibits a "B" type X-ray pattern with peaks that are both broad and weak and with the two main reflections centered at 5.5 and 17 Å 2θ angles (Colonna *et al.* 1982).

Swelling power and solubility

Legume starches have been shown to exhibit a single stage restricted swelling (Fig. 2) and low solubility patterns (Schoch and Maywald 1968; Tolmasquim *et al.* 1971; Wankhede and Ramteke 1982; El Faki *et al.* 1983*a*; Hoover and Sosulski 1985*a*). This is indicative of strong bonding forces between starch components that relax over one temperature and not at multiple temperatures as in maize (Schoch and Maywald 1968). The high intermolecular attraction between starch components may reflect an orderly arrangement of polymer chains within the starch granule, permitting close parallel alignment, thus favouring maximum interaction via hydrogen bonding. Legume starches show a wide variation in swelling power (SP) and solubility. At 90°C, the SP and solubility are in the range 11–26 and 8–25%, respectively, and, in addition, they exhibit rapid increases in SP and solubility within certain temperature ranges (60–90°C) (Schoch and Maywald 1968; Tolmasquim *et al.* 1971; Wankhede and Ramteke 1982; El Faki *et al.* 1983*a*; Hoover and Sosulski 1985*a*). Hoover and Sosulski (1985*a*) have postulated that this may be due to melting of the crystallites, which may involve a solvation-assisted helix-coil transition of their chains (Biliaderis *et al.* 1980). This in turn would result in a gain in entropy that would offset the hydrogen bonding occurring in the crystalline regions leading to increased swelling and solubility. Modification of legume starches by physical (Deshpande *et al.* 1982) and chemical (Deshpande *et al.* 1982; Hoover and Sosulski 1985*b*, 1986) methods has been shown to affect SP and solubility. Deshpande *et al.* (1982) have shown that black gram starch, subjected to heat–moisture treatments (moisture content is adjusted to levels ranging from 18 to 27% (dry weight) and

then the sample is heated for 16 h at 100°C and subsequently dried to 21°C, or a 1% (w/v) starch slurry is heated for 30 min at temperatures ranging from 60 to 95°C and then cooled and freeze dehydrated), exhibited a higher solubility and a lower SP than native starch at 95°C. This was attributed to a loss of granular structure and extensive amylose leaching. Cross-linking with phosphorus oxychloride was found to reduce the solubility (at 95°C) of black gram starch by approximately 63% (Deshpande *et al.* 1982) and the SP (at 85°C) of starches from lentil, faba bean, and field pea (Hoover and Sosulski 1986) by 35.5, 27.0, and 20.9%, respectively. Acetylation and oxidation were found to cause only marginal increases in solubility (at 95°C) of black gram starch (Deshpande *et al.* 1982).

Gelatinization

Starch, when heated in the presence of excess water, undergoes an order–disorder phase transition called gelatinization (Donovan 1979; Biliaderis *et al.* 1980) over a temperature range characteristic of the starch source. The above phase transition is associated with the diffusion of water into the granule, hydration and swelling of the starch granules, uptake of heat, loss of crystallinity, decreased relaxation time of water molecules, and amylose leaching (Stevens and Elton 1971; Lelievre and Mitchell 1975; Donovan 1979; Hoover and Hadziyev 1981). Differential scanning calorimetry (DSC) has been widely used in the study of starch gelatinization. The gelatinization temperature range, enthalpy of gelatinization (ΔH_G), and the effect of water content on gelatinization temperature could be studied by DSC. The semicrystalline nature of the starch granule led many researchers (Donovan 1979; Biliaderis *et al.* 1980; Hoover and Hadziyev 1981; Paton 1987) to use the Flory–Huggins (Flory 1953) thermodynamic equation to interpret the relationship between water content and crystallite melting observed during starch gelatinization. In the application of this equation, it was assumed that the starch–water is homogenous and gelatinization occurs under equilibrium conditions. Evans and Haismann (1982) reported that the starch–water system is not homogeneous, since it consists of individual granules suspended in a variable amount of liquid phase. Furthermore, these authors have postulated that, once starch granules reach their maximum swelling capacity, further changes in the amount of water added will not affect granule composition. Therefore, volume fractions for the Flory–Huggins equation should be based on granule composition, rather than on the composition of the entire system, particularly when considering initial gelatinization temperatures. The detection of a glass transition endotherm just prior to the gelatinization endotherm led Maurice *et al.* (1985), Biliaderis *et al.* (1986), and Slade and Levine (1988) to postulate that the process of starch gelatinization is inherently nonequilibrium in character, in that it occurs when starch granules are subjected to heat in the presence of plasticizing water in which crystallite melting is indirectly controlled by the kinetically constrained continuous amorphous environment, which was in a glassy state prior to gelatinization. Based on these observations, the above authors came to the conclusion that the applicability of the Flory–Higgins equation to starch–water systems is inappropriate. However, many researchers (Biliaderis *et al.* 1986; Paton 1987; Russel 1987) continue to use the Flory–Huggins equation, since it provides a reliable means of comparing the behavior of starches from different sources under identical experimental conditions. Of the various methods presently available for the determination of starch

TABLE 2. Proximate analysis of legume starches

Starch source	Yield of pure starch (%)	Protein (%)	Lipid (%)	Ash (%)	Amylose (%)	Iodine affinity	Source
Kidney bean	25	0.13–0.30	0.18	0.18	34.4–35.0	7.02–8.04	Hoover and Sosulski 1985*a*; Biliaderis *et al.* 1979; Yang *et al.* 1980
Northern bean	18–31	0.35–0.97	0.20–0.46	—	31.6	—	Sathe and Salunke 1981*a*
Navy bean	21–40	0.13–0.34	0.09–0.60	0.06–0.14	36	6.58–7.20	Hoover and Sosulski 1985*a*; Naivikul and D'Appolonia 1979; Schoch and Maywald 1968
Black bean	32	0.55–1.12	0.15	0.11	35.1–37.3	6.82–7.20	Hoover and Sosulski 1985*a*; Lai and Varriano-Marston 1979
Mung bean	32–43	0.12–0.28	0.17–0.50	0.18–0.27	19.5–40	5.95–6.98	Naivikul and D'Appolonia 1979; Schoch and Maywald 1968; Morrison and Liagnelet 1983
Pinto bean	27–38	0.37–0.52	0.16–0.51	0.05–0.09	25.8–30.2	—	Hoover and Sosulski 1985*a*; Naivikul and D'Appolonia 1979
Adzuki bean	21.5	0.1–0.27	0.03–0.60	0.07–0.19	21.2–34.9	6.98	Kawamura 1969; Biliaderis *et al.* 1979; Tjahjadi and Breene 1984
Moth bean	33.5	0.58	0.87	0.62	26.4	5.81	Wankhede and Ramteke 1982
Faba bean	39.9	0.49–0.52	0–0.08	0.06	31.3–42.1	6.03–5.61	Naivikul and D'Appolonia 1979; Biliaderis *et al.* 1979; Lorentz 1979; Morrison and Laignelet 1983
Horse bean	37	0.16–0.90	0.06	0.81	24–32	4.50–6.28	Lineback and Ke 1975; Vose *et al.* 1976
Lima bean	23–30	0.22–0.44	0.10	0.07–0.13	—	6.56–6.60	Schoch and Maywald 1968
Red bean	46.3	0.13	0.01	0.05	35.7	4.83	Lii and Chang 1981; Morrison and Liagnelet 1983
Lablab bean	—	0.21	0.20	0.03	30	6.05	Rosenthal *et al.* 1971
Smooth pea	40	0.52–0.70	0.01–0.1	0.07	32.5–33	6.98	Biliaderis *et al.* 1979; Biliaderis *et al.* 1981
Wrinkled pea	18–22	0.34–0.46	0.01–0.19	0.08	62.8–75.4	12.80–15.18	Schoch and Maywald 1969; Biliaderis *et al.* 1979; Colonna *et al.* 1980; Colonna *et al.* 1982; Colonna *et al.* 1981*b*
Black gram	45	—	—	—	26.65	—	Sathe *et al.* 1982
Chick pea	40	0.70–0.94	0.06	0.07	30.4–32.2	6.08	El Faki *et al.* 1983*a*; Lineback and Ke 1985
Cow pea	37	0.12–0.50	0.21–0.33	0.06	33.0	6.60	El Faki *et al.* 1983*a*; Tolmasquim *et al.* 1971
Horse gram	28	0.05	—	0.05	34.3	—	El Faki *et al.* 1983*a*
Lentil	25–42	0.17–0.53	0.05–0.23	0.13	29–45.5	6.97–9.09	Bhatty and Slinkard 1979; Shahen *et al.* 1978; Naivikul and D'Appolonia 1979; Biliaderis *et al.* 1979; Schoch and Maywald 1968

TABLE 3. Physicochemical characteristics of legume amyloses

Starch source	Iodine binding capacity	Limiting viscosity number (mL/g)	Degree of polymerization	Molecular weight	β-Amylolysis	Source
Kidney bean	20	180	1300	—	85.9	Biliaderis *et al.* 1979
Navy bean	18.48	174	1300	165 000	86.2	Naivikul and D'Appolonia 1979; Biliaderis *et al.* 1979
Black bean	22.01	—	—	—		Lai and Varriano-Marston 1979
Mung bean	19.43	251	1900	245 000	78.4	Naivikul and D'Appolonia 1979; Biliaderis *et al.* 1979
Pinto bean	—	—	—	123 000	—	Naivikul and D'Appolonia 1979
Adzuki bean	16–19.49	220	1600	—	86.8	Kawamura 1969; Biliaderis *et al.* 1979
Faba bean	19.61	188	1400	191 000	85.6	Naivikul and D'Appolonia 1979; Biliaderis *et al.* 1979
Horse bean	17.1–19.2	240–280	1800	—	82	Banks and Greenwood 1967; Biliaderis *et al.* 1981
Smooth pea	18.84–19.2	180–194	1300–1400	170 000	81.6–86.9	Biliaderis *et al.* 1979, 1980, 1981; Colonna *et al.* 1981*b*
Wrinkled pea	17.99–19.2	136–150	1000–1100	125 000	79–84.7	Biliaderis *et al.* 1979, 1980, 1981; Banks and Greenwood 1967
Lentil	19.62	188	1400	312 000	89.4	Naivikul and D'Appolonia 1979; Biliaderis *et al.* 1979

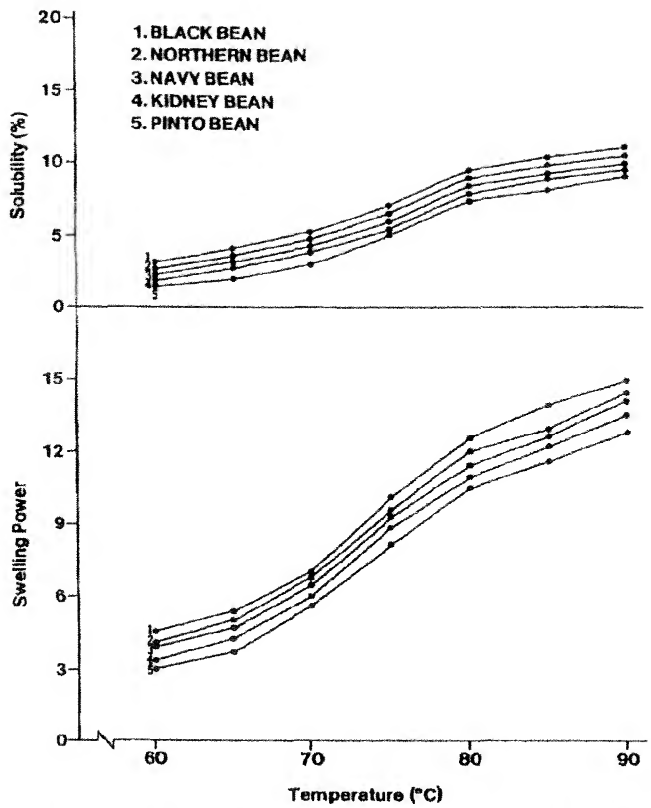

FIG. 2. The swelling and solubility patterns of legume starches. (From Hoover and Sosulski (1985*a*), with permission.)

gelatinization, such as Kofler hot stage microscope (Watson 1964), X-ray diffraction (Zobel *et al.* 1988), DSC (Donavan 1979), pulsed nuclear magnetic resonance (Lelievre and Mitchell 1975), enzymatic digestibility (Shiotsuba 1983), viscoamylography (Satahe *et al.* 1981), and small-angle light-scattering photometer (Marchant and Blanshard 1980), only the Kofler hot stage microscope and DSC have been widely used in studying the gelatinization temperatures of legume starches. The gelatinization temperatures (onset, T_o; mid-point, T_p; and end, T_c), enthalpy of gelatinization (ΔH_G), and the melting point of the most perfect crystallites (T_m^o) for legume starches are presented in Table 4. As seen in Table 4, the numerical values for T_o, T_p, and T_e, as determined by Kofler hot stage and DSC, are not in agreement in many cases. These differences are more evident with adzuki bean and lentil starches. This discrepancy may be due to varietal differences or to differences in water content of the starch–water slurries used in the experiments. The effects of annealing, heat–moisture treatment, defatting, and food ingredients on the gelatinization temperatures of legume starches have not been reported. Decreases in gelatinization temperatures and a widening of the gelatinization temperature range were found to occur when legume starches were subjected to modification by acetylation (Comer and Fry 1978; Sathe and Salunke 1981; Hoover and Sosulski 1986), hydroxypropylation (Hoover *et al.* 1988*b*), and cross-linking (Deshpande *et al.* 1982).

TABLE 4. Thermal characteristics of legume starches

Starch source	Method	T_o	T_p	T_e	Unspecified	ΔH	T_m	Source
Kidney bean	Hot stage	64	66	68		—		Biliaderis *et al.* 1980
	DSC	62	73	79		—		Yang *et al.* 1980
		67	70	76		3.6		Hoover and Sosulski 1985*a*
Northern bean	DSC[a]	63	66	70		3.0		Hoover and Sosulski 1985*a*
	Viscoamylograph	—	—	—	65.5–68.5			Sathe *et al.* 1981
Navy bean	Hot stage	68	71	74				Biliaderis *et al.* 1980
	DSC[a]	64	68	71		3.2		Hoover and Sosulski 1985*a*
Black bean	Hot stage	—	—	—	63.8–76	—		Lai and Varriano-Marston 1979
	DSC[a]	62	66	70		3.0		Hoover and Sosulski 1985*a*
Mung bean	Hot stage	64	69	76				Schoch and Maywald 1968
		63	65	69		—		Biliaderis *et al.* 1980
Pinto bean	DSC[a]	72	74	79		4.0	186	Hoover and Sosulski 1985*a*
Adzuki bean	Hot stage	64	66	68				Tjahjadi and Breene 1984
	DSC[a]	70	76	87			203	Biliaderis *et al.* 1980
Moth bean	Hot stage	62	66.5	72				Wankhede and Ramteke 1982
Faba bean	Hot stage	—	—	—	61–69			Lorenz 1979
		61	63	66				Biliaderis *et al.* 1980
Horse bean	Hot stage	61	63.5	70				Lineback and Ke 1975
Lima bean	Hot stage	70	76	85				Schoch and Maywald 1968
Red bean	Hot stage	63	66.5	70				Lii and Chang 1981
Lablab bean	Hot stage	65	70	76				Rosenthal *et al.* 1971
Smooth pea	Hot stage	49	60.5	67				Colonna *et al.* 1981*a*, 1981*b*
		65	67	69				Biliaderis *et al.* 1980
	DSC[a]	55	64	80		3.5	194	Biliaderis *et al.* 1980
		48	61	80		3.2		Colonna *et al.* 1981*a*, 1981*b*
Wrinkled pea	Hot stage	—	—	—	69–83			Schoch and Maywald 1968
	DSC[a]	—	—	—	>99			Biliaderis *et al.* 1979
					111–133	0.7		Colonna *et al.* 1982
Black gram	Hot stage	71.5	73	74				Sathe *et al.* 1982
Chick pea	Hot stage	64–70	69.5–73	74.5–78				Tolmasquin *et al.* 1971
		63.5	65	69				Lineback and Ke 1975
		60	65	75				El Faki *et al.* 1983*a*
Cow pea	Hot stage[b]	65	69	73				El Faki *et al.* 1983*a*
Horse gram	Hot stage	71	76	80				El Faki *et al.* 1983*a*
Lentil	Hot stage	58	59	61				Biliaderis *et al.* 1980
	DSC[a]	47	57	77		3.4	166	Biliaderis *et al.* 1980

NOTE: T_o, onset; T_p, midpoint; T_e, endpoint of gelatinization. ΔH, enthalpy of gelatinization. T_m, melting point of most perfect crystallites in the absence of water (calculated using the Flory–Huggins equation; Donovan 1979).
[a]DSC measurements at starch:water ratios of 1:2.
[b]Gelatinization temperature range on five varieties of cow pea.

Retrogradation

Starch granules, when heated in excess water above their gelatinization temperature, undergo irreversible swelling, resulting in amylose leaching into solution. In the presence of a high starch concentration, this suspension will form an elastic gel on cooling. The molecular interactions that occur after cooling have been called retrogradation. These interactions are found to be both time and temperature dependent. Miles *et al.* (1985) showed, with the aid of physical techniques, that amylose gelation occurs as a result of a phase separation, which produces regions that are rich and deficient in polymer and that, if the amylose concentration is sufficiently high, the region rich in polymer forms a three dimensional network. Amylose crystallization was found to be a secondary process, occurring in the region rich in polymer. Miles *et al.* (1985) and Ring *et al.* (1987) attributed the initial gel firmness during gelation to the formation of an amylose matrix gel and the subsequent slow increase in gel firmness to amylopectin crystallization. Heterogeneous acid hydrolysis of waxy maize amylopectin gels, followed by gel permeation chromatography studies on the residue (Ring *et al.* 1987), revealed that amylopectin crystallization occurs by association of amylopectin molecules with DP = 15. The crystallization of amylopectin was shown to be reversible at temperatures below 100°C, whereas the initial gelation and crystallization of amylose was irreversible even at 100°C (Miles *et al.* 1985; Ring *et al.* 1987). This indicates a greater degree of molecular interaction in the latter. X-ray diffraction and shear modules studies on various starches (Orford *et al.* 1987) showed that the initial rates of development and stiffness of gels followed the order smooth pea > maize > wheat > potato, while the long-term increase followed the order smooth pea > potato > maize > wheat. The latter process was found to be more important at high starch concentrations. Differences in the fine structure of amylopectins, and also in the extent of association between starch components, may account for the above observed retro-

gradation tendencies. The freeze–thaw stability of starch gels is an important characteristic of food starches. This stability is determined by gravimetric measurement of the water exuded (syneresis) from a gel after it has been frozen and thawed. This exudation occurs owing to the reassociation of linear starch molecules (retrogradation). This high retrogradation tendencies of legume starches was also shown by Hoover and Sosulski (1985*a*) and Tjahjadi and Breene (1984) by measurement of the degree of syneresis during low temperature storage. The high degree of synesesis makes native legume starches unsuitable for use in foods requiring low temperature storage. However, modification by acetylation and hydroxypropylation was found to significantly decrease the extent of syneresis to levels that may be acceptable to food processors (Hoover and Sosulski 1986; Hoover *et al.* 1988*b*).

Rheology

Rheological measurements on starches are routinely carried out in the food industry to test the ability of the starches to function effectively as thickening agents. Compared with cereal and tuber starches, information on the rheological characteristics of legume starches is limited. Although the Brabender/Visco/Amylogram (BVA), Ottawa starch viscometer (OSV), and rotational viscometers have been utilized to examine starch rheology, only the BVA (Schoch and Maywald 1968; Lineback and Ke 1975; Yang *et al.* 1980; Lii and Chang 1981; Sathe and Salunke 1981; El Faki *et al.* 1983*a*; Tjahjadi and Breene 1984; Hoover and Sosulski 1985*a*; Abbas *et al.* 1986; Paredes-Lopez *et al.* 1988) and OSV (Bhatty and Slinkard 1979; Doublier 1987) have been used in the case of legume starches. Some researchers (Steeneken 1989; Muhrbeck and Eliasson 1987) are of the opinion that rheological properties of starches cannot be properly assessed using the BVA, since the viscosity measurements are made under nonlaminar flow conditions and, in addition, the starch paste is subjected to both thermal and mechanical treatment, thus making it difficult to relate viscous behaviour to only one of these parameters. However, the BVA is useful in comparing the overall rheological behaviour of starches (from various sources) under identical conditions (concentration, pH, and shear). In rheometers, utilizing the cone and plate or coaxial cylinder geometry, the thermal treatments are separated from shear effects. There is, thus, a need to extend the use of rheometers to investigate the rheology of gelatinized legume starch suspensions under well-defined flow regimes and to compare these results with those obtained from the BVA.

In the BVA, all legume starches show the absence of peak viscosity, constant or increasing viscosity during the holding period (at 95°C), a high setback and a constant cold paste viscosity during agitation at 50°C. Such a behavior is indicative of a high degree of interaction between starch components. Most legume starches exhibit a type C (restricted swelling) viscosity pattern (Schoch and Maywald 1968). However, tepary bean (Abbas *et al.* 1986) and mung bean (Schoch and Maywald 1968) starches exhibit a mixed viscosity pattern resembling type C at low concentrations and type B (moderate swelling) at higher concentrations. A type D (highly restricted swelling) viscosity pattern, however, is shown by wrinkled pea starch (Schoch and Maywald 1968). Decreases in initial pasting temperatures and increases in the 95°C viscosity, shear stability, and set-back were observed in legume starches subjected to acetylation (Comer and Fry 1978; Hoover and Sosulski 1985*b*). Cross-linking of legume starches with phosphorus oxychloride was found to decrease their initial pasting temperatures and the 95°C viscosities, but conferred great stability against viscosity breakdown during the holding period at 95°C, and also increased the extent of setback (Hoover and Sosulski 1986). The effect of cross-linking in reducing the extent of amylose exudation and in increasing granule stability has been shown by scanning electron microscopy (Hoover and Sosulski 1986) of samples taken from the BVA at various time intervals.

Digestibility

Legume starches in foods have been found to be more digestible than potato or high amylose maize starch but less digestible than cereal or cassava starch (Dreher *et al.* 1984; Hoover and Sosulski 1985a*a*; Ring *et al.* 1988). Ring *et al.* (1988) have shown that, during a 24-h digestion period with porcine pancreatic α-amylase, the percentage hydrolysis of native starches from wheat, maize, smooth pea, and potato were 100, 95, 67 and 15%, respectively. A similar observation was made by Hoover and Sosulski (1985*a*) who showed that, during a 6-h digestion period, corn starch was hydrolyzed to the extent of 75% by porcine pancreatic α-amylase, whereas the corresponding values for legume starches belonging to the species *Phaseolus vulgaris* ranged from 26 to 35%. These differences in the *in vitro* digestibility of native starches among and within species have been attributed to the interplay of many factors such as starch source (Ring *et al.* 1988), granule size (Snow and O'Dea 1981; Ring *et al.* 1988), starch–protein interactions (Würsch *et al.* 1986), amylose/amylopectin ratio (Dreher *et al.* 1984; Hoover and Sosulski 1985*a*; Holm and Björck 1988; Ring *et al.* 1988), percentage of retrograded starch (Ring *et al.* 1988), extent of molecular association between starch components (Dreher *et al.* 1984; Hoover and Sosulski 1985*a*; Holm and Björck 1988), physical distribution of starch in relation to dietary fiber components (Rao 1969; Snow and O'Dea 1981; Dreher *et al.* 19184), antinutrients (Thompson and Gabon 1987; Thompson and Yoon 1984), α-amylase inhibitors (Puls and Keup 1973), degree of crystallinity (Dreher *et al.* 1984; Hoover and Sosulski 1985*a*; Ring *et al.* 1988), amylose chain length (Jood *et al.* 1988), amylose–lipid complexes (Holm *et al.* 1983), and the influence of drying methods and storage conditions (Kayisu and Hood 1979). It is likely that differences in the observed digestibility of starch samples could also be attributed to differences in the α-amylase activity of enzyme preparations. As seen in Table 5, the extent of *in vitro* digestibility of black bean and chick pea starches was found to depend on the source of α-amaylase (Rosenthal and Nakamura 1972; El Faki *et al.* 1983*b*; Hoover and Sosulski 1985*a*; Soccorro *et al.* 1989). There is a need to extend this study to other legume starches as well. Changes in the molecular size of hydrolytic products during α-amylolysis of legume starches and their components (amylose and amylopectin) also needs investigation.

Studies of partially digested raw starch granules (El Faki *et al.* 1983*b*; Ramadas Bhat *et al.* 1983; Hoover and Sosulski 1985*a*; Tharanthan and Ramadas Bhat 1988) have been made using scanning electron microscopy. Unlike in digested cereal starches, which exhibit numerous pinholes on the surface layer (Fig. 3) with those pores penetrating into the granule (Dronzek *et al.* 1972; MacGregor and Ballance 1980; Hoover and Sosulski 1985*a*), legume starches (Ramadas Bhat *et al.* 1983;

TABLE 5. *In vitro* amylolysis of legume starches

Starch source	Source of α-amylase	Reaction time (h)	Degree of hydrolysis (%) (maltose equivalents)	Source
Black gram	Type XA from *Asperigillus niger*	1	Not detected	Tharanathan and Ramadas Bhatt 1988
Chick pea	Human saliva	3	24.3	El Faki *et al.* 1983*b*
	Bacillus subtilis	2	41	Rosenthal and Nakamura 1972
Cow pea	Human saliva	3	17.7	El Faki *et al.* 1983*b*
Horse gram	Human saliva	3	15.3	El Faki *et al.* 1983*b*
Lablab	*Bacillus subtilis*	2	30	Rosenthal and Nakamura 1972
Jack bean	*Bacillus subtilis*	2	25	Rosenthal and Nakamura 1972
Kidney bean	Pancreatic porcine	6	31.4	Hoover and Sosulski 1985*a*
Black bean	Pancreatic porcine	6	34.8	Hoover and Sosulski 1985*a*
	Pancreatic porcine	3	49.5	Soccorro *et al.* 1989
	Pancreatic porcine	3	30.1	Soccorro *et al.* 1989
	Pancreatic porcine	3	66.1	Soccorro *et al.* 1989
Smooth pea	Pancreatic porcine	5	71.4	Biliaderis 1982
	Pancreatic porcine	24	67	Ring *et al.* 1988
Navy bean	Pancreatic porcine	6	32	Hoover and Sosulski 1985*a*
Northern bean	Pancreatic porcine	6	29	Hoover and Sosulski 1985*a*
Pinto bean	Pancreatic porcine	6	25.2	Hoover and Sosulski 1985*a*

FIG. 3. SEM micrograph of an α-amylase hydrolyzed wheat starch granule. (From Dronzek *et al.* (1972), with permission.)

Hoover and Sosulski 1985*a*) generally exhibit only roughened surfaces indicating surface erosion (Fig. 4). However, El Faki *et al.* (1983*b*) reported that salivary α-amylase attacks cowpea and horse gram starch causing pitting as well as surface erosion resulting in onion-type layering of degraded granules. Attempts have been made to improve the *in vitro* digestibility of legume starches by cooking (Rao 1969; Kumar and Venkataraman 1976; Fleming 1982; Jood *et al.* 1988), soaking (Jood *et al.* 1988; Kataria and Chauhan 1988), and germination (Kumar and Venkataraman 1976; Jaya and Venkataraman 1980; Jyoti and Reddy 1981; Jood *et al.* 1988; Kataria and Chauhan 1988). Kataria and Chauhan (1988) showed that soaking mung beans for 18 h increased starch digestibility by 40%. However, soaking of chick pea or black gram for 12 h was found to increase digestibility only marginally. Ordinary cooking of soaked and unsoaked seeds increased starch digestibility of mung bean (Kataria and Chauhan 1988) by 623 and 555%, respectively. The corresponding values were 207 and 145% for black gram (Jood *et al.* 1988) and 163 and 99.6% for chick pea (Jood *et al.* 1988). Autoclaving of soaked and unsoaked mung beans (Kataria and Chauhan 1988) increased starch digestibility by 987 and 808%, respectively. Similar findings were reported by Jood *et al.* (1988) on soaked, autoclaved chick pea and black gram where the increase in diges-

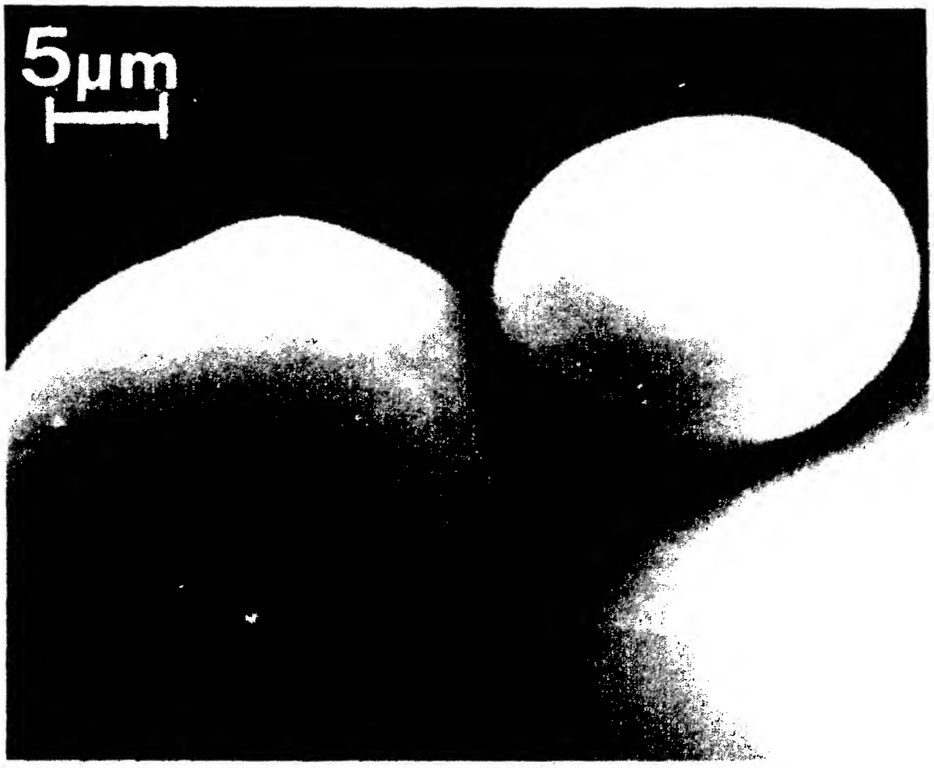

FIG. 4. SEM micrographs of α-amylase hydrolyzed (1 h) kidney bean starch granules. (From Hoover and Sosulski (1985*a*), with permission.)

tibility amounted to 453 and 580%, respectively. The differences in starch digestibility during cooking and autoclaving may be due to differences in the extent of swelling, gelatinization, and destruction of the crystalline structure of the starch granule.

The digestibility of starch from germinated seeds of mung bean (Kumar and Venkataraman 1976), chick pea (Jood *et al.* 1988), and black gram (Jood *et al.* 1988) was found to be three times the digestibility of ungerminated seeds. Cooking was found to increase the digestibility of starches from germinated beans (Kumar and Venkataraman 1976; Jood *et al.* 1988). The digestibility of starches from cooked, germinated chick pea, cowpea, and green gram was found to be two to four times higher than that seen with uncooked germinated seeds (Kumar and Venkataraman 1976). The corresponding value for black gram and chick pea was 1.3 (Jood *et al.* 1988).

El Faki *et al.* (1983*b*) investigated the *in vivo* digestibility of starches from chickpea, cowpea, and horse gram. They estimated the *in vivo* starch digestibility compared with that of corn starch by determining the increase in weights and contents of small intestine and caecum, apparent digestibility, and faecal weights of rats. The *in vivo* digestibility of the legume starches was lower than that of corn. The weight gain and apparent starch digestibility followed the order corn > chickpea > horse gram, while the faecal weight and weights of organs and their contents followed the order horse gram > cowpea > chickpea > corn. The caecum pH of rats fed legume starches was distinctly acidic in comparison to that of rats fed corn starch, which was neutral. The acidic pH of the caecum was attributed to the formation of carbon dioxide and organic acids as the result of fermentation of indigestible carbohydrates. Scanning electron microscopy revealed that, of the three legume starches tested, chickpea starch granules appeared to be more digestible, both by *in vivo* and *in vitro* systems (El Faki *et al.* (1983*b*). These authors have postulated that the actual composition of starch *per se* may decide the nature, type, and degree of amylolysis in *in vitro* systems, while in *in vivo* systems the combined action of different enzymes, including those from microorganisms, and changes in pH may bring about significant hydrolysis of native starch granules. Fleming and Vose (1979) and Fleming (1982) investigated the *in vivo* digestibility of several legume starches by estimating the starch content of the rat caecum. These authors found that, with the exception of high amylose wrinkled pea starch, all other legume starches were 100% digestible. However, the legume starches reduced the digestibility of casein protein by 3–4%. Fleming (1982) showed that drying and cooking methods do not improve *in vivo* starch digestibility. This was in contradiction to the *in vitro* studies. This seems to indicate that the mechanism of digestion of legume starches under *in vitro* and *in vivo* conditions needs to be investigated more thoroughly. Such a study is of importance owing to the recent interest in including legumes as a part of the diet of diabetic patients (Jenkins *et al.* 1980).

Modification of legume starches by substitution has been shown to decrease their *in vitro* digestibility by porcine pancreatic α-amylase (Hoover and Sosulski 1985*b*, 1988*b*). Decreases of 23.8, 5.8, and 5.2% were observed in acetylated (degree of substitution ~0.05) starches of black bean, navy bean, and pinto bean, respectively (Hoover and Sosulski

1985*b*). After a 6-h hydrolysis with α-amylase, both native and acetylated black bean starch granules were found to exhibit roughened surfaces. However, the number of granules attacked by α-amylase was very much less in the latter (Hoover and Sosulski 1985*b*). These authors have postulated that the reduced rate of hydrolysis seen with acetylated starches may involve both steric and electronic factors. Similar decreases in hydrolysis have also been observed with hydroxypropylated field pea starch (Hoover *et al.* 1988*b*). Increasing the level of molar substitution (MS) was found to cause an initial decrease in digestibility followed by increases at MS levels beyond 0.08. Scanning electron microscopy revealed that more starch granules were degraded at MS = 0.12 than in the native starch (Hoover *et al.* 1988*b*). The above authors attributed the initial decrease in hydrolysis, at low levels of MS, to the steric hindrance imposed by the bulky hydroxypropyl groups on C2 towards the action of the catalytic carboxylate anion of α-amylase on the glycosidic bond. The subsequent increase in hydrolysis at high levels of MS (0.12) was attributed to an increase in the SP of the amorphous region of the starch granules. At high MS levels, SP was found to be more important than steric factors in influencing enzymic activity. Legume starches cross-linked with phosphorus oxychloride were found to exhibit only marginal decreases in the extent of hydrolysis (Hoover and Sosulski 1986). Recently, there has been much interest in characterizing resistant starch (RS), since it behaves as a dietary fiber, in escaping digestion in the small intestine, but being readily fermented in the colon by microorganisms (Asp *et al.* 1986; Björck *et al.* 1986, 1987; Englyst and Cummings 1985; Englyst and MacFarlane 1986; Siljestrom and Asp 1985; Wyatt and Horn 1988). Resistant starch formation has been shown to occur only during certain food-processing operations such as baking and autoclaving (Russell *et al.* 1989; Siljestrom and Asp 1985). Russell *et al.* (1989) have shown that purified wheat RS was largely carbohydrate of which the sugar composition was greater than 96%. Gel permeation chromatography has revealed that wheat RS exhibits a distribution of low molecular weight glucans with a DP in the range 60–65 (Russell *et al.* 1989; Siljestrom *et al.* 1989). Furthermore, incubation of wheat RS with isoamylase was found to cause no changes in the apparent DP, indicating that RS was composed of short stretches of linear $(1 \rightarrow 4)$-α-glucans with amylose molecules (Russell *et al.* 1989) that preferentially crystallize from solution during cooling of cooked starch pastes and are free of molecular interactions to move into crystalline arrays (Russell *et al.* 1989). The increase in RS content during autoclaving has been attributed to the mobilization of starch polymers owing to granular swelling, which results in separation followed by crystallization of the amylose chains. Sievert and Pomeranz (1989) have also shown, with the aid of thermoanalytical, enzymatic, and microscopic methods, that RS is mainly derived from recrystallized amylose. Although amylose crystallites are responsible for RS formation, it is still not clear when these crystallites are formed. For instance, Russell *et al.* (1989) have postulated that, since RS is formed during the interaction of amylolytic enzymes with starch, it is possible that short chain $(1 \rightarrow 4)$-α-glucans could crystallize as separate molecules mobilized by release from the amylose, rather than as thermally mobilized chains within the amylose macromolecules. Most of the structural studies on RS have been on starches from wheat, amylomaize, and waxy maize. RS from legume starches, with the exception of pea starch (Ring *et al.* 1988), have received only scant attention. This is rather surprising since their high amylose content and wide variations in the magnitude of bonding forces (between starch components) and retrogradation rates may provide more insights into how these factors could affect the mechanism and extent of formation of RS.

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